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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Delcourt, R. 1967. Démonstration par l'électrophorèse en gel d'acryle de la spécificité du bleu Luxol en tant que réactif des phospholipides (Demonstration by acrylic gel electrophoresis of the specificity of Luxol blue in the reaction with phospholipid). Comptes rendus des séances de la Société de Biologie (Proceedings of the sessions of the Society of Biology) vol. 161, No. 6, pp. 1480-1481.

The phtalocyenides are blue dyes containing a metallic atom in the center of a heterocylic nucleus. The metallic ion is from the oxide used as a catalyst in the synthesis of the dye (Cram and Hammond (1)). Pearse (2) demonstrated that these dyes bind to phospholipid structures such as mycin and red blood cell stroma. In vitro, these same dyes form insoluble complexes with phospholipids containg choline. Lison (3) reported, without any remarks, a limitation in the specificity of this dye for phospholipids. Salthouse has demonstrated the formation of stoichiometric complexes between Luxol blue and phospholipids containing choline or serine.

We have studied the action of Luxol fast blue on blood proteins and their fractions isolated by acrylic gel electrophoresis using a method we previously described (Delcourt (5)).

1. Action on Fixed Proteins. Several spottings of serum on Whatmann No. 1 paper were stained, after dessication, with an alcoholic solution of Luxol fast blue. The excess stain was eluted from the paper using 5 % acetic acid. In addition, the stain fixed to the serum spots was not soluble in a number of conventional solvents, There was, therefore, co-precipitation.

2. Action on Blood Serum. A saturated solution of stain in cellosolve was prepared. The addition of less than 40 µl of this solution to 1 ml of serum stained it without any precipitation occurring. When 40 µl or more were used, precipitation occurred. This precipitate was insoluble in water and in the usual solvents. The precipitate was quite certainly the result of the formation of an insoluble complex. There was a negative test for free copper in an aqueous solution of the stain; the addition of sodium hydroxide to the serum mixture did not give the characteristic biuret color.

3. Thin-Layer Chromatography. Thin-layer chromatography did not show any migration or any fractionation.

4. Acrylic Gel Electrophoresis. This confirmed the specific fixation of the lipoproteins: (A) the dye alone, in aqueous solution, migrated rapidly towards the cathode without leaving any material along its path of migration; (B) lipid-free solutions of Cohn fractions migrated without retaining any of the dye; (C) a solution of thromboplastin, probably denatured by phenol but still maintaining intact biological activity, was separated into several bands which stained with Luxol blue and whose lipid nature was confirmed by staining with acetyl Sudan blue; (D) several human and animal sera, pre-stained with Luxol blue, gaive from four to five fractions whose color reactions were similar to those for alpha-lipoproteins.

Discussion. We have confirmed the preferential fixation of Luxol blue to lipoproteins rich in phospholipids, such as alpha-lipoproteins and thrombo-plastin, and the complete absence of staining with other native or lipid-free proteins. The mode of fixation does not occur at the level of the active groups responsible for electrophoretic mobility of lipoproteins, as

shown by the simultaneous electrophoresis of serum prestained with Sudan blue, Nile blue, and Luxol fast blue. It is not the copper atom that interacts and the method of coupling suggested by Pearse is still possible - the formation of a salt of the phospholipid with the dye.

References

(1) D.J. Cram and G.S. Hammond, Chimie organique, Presses Univ. Laval, Quebec, 1963, p. 680

(2) A.G. Pearse, Jour. Path. Bact., 1955, 70: 554.

(3) L. Lison, Histochimie et cytochimie animales, Gauthier-Villars, Paris, 1960, p. 524.

(4) T.N. Selthous, Nature, 1962, 195: 187.

(5) R. Delcourt, Protides of the Biological Fluids, 15th Colloqium, Bruges, 1977. To be published.